Atypical Aeromonas salmonicida vapA type V and Vibrio spp. are predominant bacteria recovered from ballan wrasse (Labrus bergylta A.) in Scotland

Citation for published version:
Papadopoulou, A, Wallis , T, Ramirez Paredes, JG, Monaghan , SJ, Davie, A, Migaud, H & Adams, A 2020, 'Atypical Aeromonas salmonicida vapA type V and Vibrio spp. are predominant bacteria recovered from ballan wrasse (Labrus bergylta A.) in Scotland', *Diseases of aquatic organisms*, vol. 140. https://doi.org/10.3354/dao03489

Digital Object Identifier (DOI):
10.3354/dao03489

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Diseases of aquatic organisms

Atypical *Aeromonas salmonicida vapA* type V and *Vibrio* spp. are predominant bacteria recovered from ballan wrasse (*Labrus bergylta* A.) in Scotland

A. Papadopoulou[1,2], T. Wallis[3], J. G. Ramirez – Paredes[3], S. J. Monaghan[1], A. Davie[1], H. Migaud[1] and A. Adams[1*]

[1]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK alexandra.adams@stir.ac.uk

[2] The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, UK

[3]Ridgeway Biologicals Ltd, Units 1-3 Old Station Business Park, Compton, Nr Newbury G20 6NE UK

*Correspondence author: apapadou@exseed.ac.uk

Running page head: Atypical *Aeromonas salmonicida* in Scottish ballan wrasse (*Labrus bergylta* A.)

Abstract

Healthy and / or moribund farmed and wild ballan wrasse, *Labrus bergylta* (>0.5 to 900 g) were sampled from hatcheries (n= 2) and Atlantic salmon cage sites (n= 8) in Scotland between February 2016 and October 2018. Less than half of the sampled individuals (n= 43, 32.3 %) had been vaccinated (autogenous polyvalent vaccine; dip and / or injection) against atypical furunculosis (type V and VI) while 20 (15.0 %) fish were not vaccinated and the rest (70 individuals, 52.7 %) were of unknown vaccination status. Swab samples from skin lesions, gill, liver, spleen and kidney were inoculated onto a variety of bacteriological agar plates and bacteriology identification and sequencing analysis was performed on significant bacterial colonies. Atypical *Aeromonas salmonicida* (a*As) vapA* type V was the predominant bacterial species (70/215 bacteria isolates; 32.5 % of bacteria samples – 43/117 positive individual fish; 36.8 %) isolated in this survey followed by *Vibrio* species which were the most geographically prevalent bacteria. *Photobacterium indicum/profundum* was also isolated from *L. bergylta* for the first time during this study. The collection of these bacterial isolates provides useful information for disease management. Identifying the a*As* isolates involved in disease in ballan wrasse could provide vital information for improving / updating existing autogenous vaccines.

Key words: atypical *Aeromonas salmonicida*, ballan wrasse, health survey, cleaner fish

1. INTRODUCTION

Ballan wrasse (*Labrus bergylta* Ascanius, 1767) and lumpsucker (*Cyclopterus lumpus* Linnaeus, 1758) are two cleaner fish species that have been intensively used by the Atlantic salmon (*Salmo salar* L.) farming industry as an alternative means to control sea lice (*Lepeophtheirus salmonis* Krøyer, 1837). The latter is an ectoparasite of the Northern hemisphere that causes major economic and welfare implications on this aquaculture industry (Treasurer 2012, Skiftesvik et al. 2013). Initially wild wrasse species (cuckoo; *Labrus mixtus* L., corkwing; *Symphodus melops* L., goldsinny; *Ctenolabrus rupestris* L. and rockcook; *Centrolabrus exoletus* L.) were used in salmon cages. However, the demand for fish and biosecurity concerns regarding the health status of wild deployed cleaner fish along with sustainable supply of wild wrasse on cage sites has led to rearing of ballan wrasse in Scotland since 2010.

Ballan wrasse are known to be susceptible to bacterial (*e.g.* atypical strains of *Aeromonas salmonicida* (a*As*) and *Vibrio* spp.) (Biering et al. 2016, Gulla et al. 2016, Brooker et al.

2018), parasitic (*e.g.* amoebic gill disease (AGD) (Karlsbakk et al. 2013) and viral (*e.g.* piscine myocarditis virus; PMCV) (Scholz et al. 2018) diseases. Various *Vibrio* species (*Vibrio anguillarum*, *V. ordalii* and *V. splendidus*) have also been isolated from diseased (symptomatic to vibriosis) ballan wrasse but only *V. anguillarum* originally isolated from Atlantic salmon caused high mortalities (up to 60%) in ballan wrasse under experimental conditions (Biering et al. 2016). Thus, pathogenicity of *Vibrio* species in ballan wrasse is not clear. Atypical strains of the bacterium *Aeromonas salmonicida (As)* have also been reported during mortality events of ballan wrasse in Norway (Bornø and Gulla 2016). An additional, outer membrane - the paracrystalline surface protein (A-layer protein) - plays an important role in the infection of the host as well providing protection for the bacterium by resisting host response processes (Udey & Fryer 1978, Munn et al. 1982, Kay & Trust 1991, Daly et al. 1996). The gene that encodes this protein is known as the virulence array protein A (*vapA*) and 23 A – layer (*vapA*) types of *As* were identified by sequencing the hypervariable region of the gene (Gulla et al. 2016, Gulla et al. 2019). Furthermore, type V and VI found to be related with cleaner fish species *L. bergylta* and *C. lumpus* in Scotland and Norway (Gulla et al. 2016, Gull et al. 2019). Cohabitation and intraperitoneal (i.p.) injection with a*As* (one strain of each type V and VI used) successfully induced disease and morbidities during experimental conditions (Biering et al. 2016). Specifically, type V was found to cause the highest morbidities, suggesting that atypical strains are virulent to the species *L. bergylta* (Biering et al. 2016).

Information related to mortality events including causative agents / pathogens of cleaner fish such as ballan wrasse in Scotland is limited (Treasurer 2012). Bacterial disease outbreaks have been speculated to be related with a*As* on commercial sites in Scotland but there are very few reports available. Prevention of disease outbreaks through vaccination is needed for the species *L. bergylta* in order to improve their welfare in aquaculture and to enable their efficient performance as cleaner fish in salmon pens. Health screening and characterisation of these bacterial pathogens is essential for successful vaccine formulation. Thus, in the current study, a real-time health survey was conducted to determine the bacterial pathogens present in both farmed ballan wrasse hatcheries and Atlantic salmon cage sites (wild and farmed fish) in Scotland between February 2016 and October 2018, in order to identify the most prevalent bacterial pathogens of ballan wrasse.

2. MATERIALS AND METHODS

2.1. Bacterial identification

Healthy and / or moribund farmed and wild ballan wrasse (> 0.5 to 900 g, n= 133) were sampled from hatcheries (n= 2) and Atlantic salmon cage sites (n= 8) in Scotland between February 2016 and October 2018. Less than half of the sampled individuals (n= 43, 32.3%) had been vaccinated with an autogenous polyvalent vaccine (Ridgeway Biologicals Ltd) which included atypical furunculosis (type V and VI). From those, 42 individuals were originating form site A and had been vaccinated by two immersions (prime; ca. 0.5 g and booster vaccination; ca. 2 g) and / or injection at ca. 15 g and all the fish in the batches from which these individuals originated from had been vaccinated with the same practice. There was one more individual that had been vaccinated however, no information has been provided and whether the rest of the cleaner fish on site had been vaccinated. Furthermore, 16 (12.0%) fish were unvaccinated and the rest of the individuals (n=74, 55.6%) were of unknown vaccination status (farmed or wild origin). Swab samples from skin lesions, gills, liver, and kidney were inoculated onto Marine Agar, Tryptone Soya Agar (TSA), and TSA + 5% Defibrinated Horse Blood + 1.5% NaCl, and incubated at 22°C for 24 – 72h for primary bacterial isolation. Pure colonies were then picked on the basis of morphology, predominance and prevalence, streaked onto fresh plates and incubated, as described before, for purification. Passaged isolates were then tested by Gram’s staining (bioMerieux) and

Catalase (catalse reagents. VWR UK)/ Oxidase (oxidase strips, Oxoid UK) tests for purity confirmation and primary identification.

2.2. Molecular analysis

Bacterial DNA was extracted using genesig® Easy DNA/RNA Extraction Kit (Genesis) according to the manufacturer's instructions. Bacterial species identification was performed on the samples by targeting the subunit B protein of DNA gyrase (topoisomerase type II) – *gyrB* gene (Yamamoto et al. 2000) and V3-V4 hypervariable region of the *16S rRNA* gene (Klindworth et al. 2013) (Table 1). PCR reactions consisted of each primer at 10 μM, 1 unit of GoTaqG2 master mix (Promega), 5 μL of DNA sample and milliQ water to reach a final reaction volume of 25 μL. The following thermal cycling conditions were used in G-storm thermocycler: 1 cycle at 95°C for 5 min, 35 cycles at 95°C for 30 sec, 55°C (gyrB) and 44°C (16 rRNA) for 30 sec and 73°C for 1 min, followed by 1 cycle at 73°C for 7 min. The PCR product was then purified using QIAquick PCR Purification Kit (Qiagen, Germamy) as described by the manufacturer and 3.5 μL of the clean-up were mixed with 2.5 μL of each of the forward and reverse primers in a separate nuclease free Eppendorf tube and 1.5 μL of nuclease free water to reach a total volume of 7.5 μL. Products sent for sequencing to GATC (Eurofins) and obtained sequences were compared to known sequences using an in silico nucleotide alignment tool 'BLAST' (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Isolates that were recognised as presumed a*As* by the naked eye; small, friable colonies, non-motile coccobacilli (prior to 16S confirmation) or by PCR testing (16S) were then confirmed to be a*As* using the A-layer membrane - *vapA* primer sets (Gulla et al. 2016) to determine the *vapA* strain type (Table 1) as described by Gulla et al. (2016). The PCR product was then purified using QIAquick PCR Purification Kit (Qiagen, Germamy) and samples mixed with forward and reversed primers as described above and sent for sequencing to GATC (Eurofins). Sequences were analysed with Clustal Omega at EMBL-EBI (https://www.ebi.ac.uk/) against the published type strain sequences.

3. RESULTS

Among 327 samples (n= 133 individual fish) collected from all sites, 192 (n= 117 individual fish) had visible colonies which were identified using biochemical (Gram staining, catalase and oxidase test) and molecular (*gyrB* and *16S rRNA* sequencing, a*As vapA* assay) analysis (Table 2).

Atypical *As* was detected in 70 (43/117 positive individual fish; 36.8%) out of 215 bacteria isolates (32.5 % of bacteria samples). Following *vapA* gene screening the majority of the a*As* colonies belonged to *vapA* type V with the exception of 2 individuals that were positive to type VI from sites E and J. Atypical *As* were the most prevalent of the pathogenic bacteria species during this survey followed by *Vibrio* spp. and *Aliivibrio* spp.– *Vibrio ichthyoenteri. Vibrio splendidus, Vibrio tasmaniensis Aliivibrio logei, Aliivibrio salmonicida, Allivibrio finisterrensis*– (69/215; 32.1% vibrio isolates and 55/116; 47.4% positive individuals) (Figure 1 and 2). The bacteria prevalence per site is shown in Figure 1 and 2. Atypical *As* is most prevalent in sites A, C and E; note that site E is not presented in a pie chart as a*As* (2 isolates *vapA* V) were the only bacteria recovered from a single individual in a single sampling event, while *Vibrio* spp. were the most prevalent in sites B, I and J (Figure 1 and 2). The majority of a*As vapA* type V had been isolated from liver (25) and kidney (32), while the least a*As* recovery was noted from fin (5), skin (4) and gill (2) samples. Also the a*As vapA* type VI isolates (2) were from skin, liver and kidney of deployed ballan wrasse. The 54.3% of the a*As* (*vapA* type V and VI) isolates recovered were from vaccinated fish (21 /43 individuals; 47.7%) and the majority (20 / 21) were originating from site A. Nearly half of the vaccinated individuals (20/42; 47.6 %) were positive for the bacterium (a*As*). Furthermore, 8.6% of the a*As* were from non vaccinated individuals (4 / 43; 9.1%) and the remaining a*As* isolates (37.1%) were recovered from fish with unknown vaccination status (19 / 43; 43.2%).

Apart from a*As* another 100 (46.0 %, 82/117 individuals; 70.0%) isolates were identified and could potential be pathogenic in farmed ballan wrasse as they are known fish pathogens. These were *Aliivibrio finisterrensis*, *Aliivibrio* sp., *Aliivibrio salmonicida, V. anguillarum*, *Vibrio atlanticus*, *V. ichthyoenteri, V. lentus, , V. splendidus, V. tasmaniensis, T. ovolythicum, T. soleae, T. diecentrachi* and *Pseudomonas putida, Pseudomonas psychrophila, Pseudoalteromonas* sp. and *M. viscosa.* The above were recovered from gills, fins, liver, spleen and head kidney except *T. ovolythicum, T. soleae, T. diecentrachi*, *Pseudoalteromonas* sp. and *Moritella viscosa* which were isolated only in at least one of the following skin lesions, gills and / or fins.

No external disease signs were noted on the fish with a few exceptions. The majority of fish sampled from site A had fin rot and fish were lethargic. Internally, in some cases, the following clinical signs were observed: granulomas in the liver and/or kidney, ascites and empty gut which in some individuals was red. A suspected atypical As outbreak was active during the samplings on site A. Vaccination status of the fish did not significantly affect external or internal gross pathology for site A. Furthermore, a single wild individual from site C had a heavy skin ulcer in the flank and 3 individuals sampled at site D had pale gills, empty guts and granulomas in the organs. Co-occurrence of a*As* and *Vibrio* spp. was noted for sites A, B and C in 5, 2 and 1 individual, respectively. Bacteriology analysis also showed that the individual from site C was positive for *V. splendidus* in the liver and *P. indicum* on the skin and kidney, while from three individuals (site D) *Vibrio* spp. and *Shewanella* sp. was isolated from liver and *P. indicum* from kidney.

Non-pathogenic bacteria also present in the samples included: *Arthrobacter* sp., *Bacillus simplex*, *Chryseobacterium* sp., *Colwelia* sp., *Glaciecola punicea, Leucothrix mucor, Oleispira antartica, Pianococcus* sp., *Planococcus* sp., *P. indicum, Phot. phosphoreum, Phot. profundum, Photobacterium* sp., *Polaribacter irgensii, Polaribacter* sp., *Pseudoalteromonas marina, Pseudomonas fragi, Psychrobacter marinicola, Psychrobacter* *nivimaris, Psychrobacter glacincola, Shewanella* sp*, Staphylococcus warneri, Vibrio tapetis*. *Photobacterium indicum* was also isolated from 4 locations, sites B, C, D and J with prevalence of 21.4% (3 / 14 individuals), 26.3% (5 / 19 individuals), 20.0% (1 / 5 individuals) and 22.2% (2 / 9) respectively. The sequencing data in comparison with BLAST searches gave high species similarity (97-99%) for all the above sequences.

4. DISCUSSION

In this study a bacteriology health survey was conducted at ballan wrasse hatcheries (n= 2) and Atlantic salmon sea sites (n= 8), where wild and farmed wrasse have been deployed in Scotland, for more than 2.5 years. The majority of the sampled ballan wrasse did not have external sign of diseases with few exceptions for fish from site A, a single wild individual sampled at site C and 3 individuals sampled at site D. The predominant pathogenic bacterial species identified after bacteriology assessment and sequencing analysis (16S rRNA and *gyrB*) was a*As vapA* type V. In corroboration with Gulla et al. (2015) a*As* type V appears here to be the most predominant strain in Scotland whereas strain type VI appears to be mainly in Norway.

Atypical strains of *As* were isolated from 6 out of 10 sites that took part in this health screening survey and the bacterium was the most prevalent in 4 out of 10 sites. The results from this survey suggest that a*As* was the most prevalent bacterial species at these sites between February 2016 and October 2018. It is worth noting that the a*As vapA* type VI isolates in this survey originated from two deployed individuals in sea cages and were speculated to be related to a secondary infection following immune suppression and /or be indicative of virulence adaptation of type VI against the host. Although currently, antibiotic treatments are successfully applied for controlling disease outbreaks in hatcheries and cage sites, *As* is known carry plasmids linked with antibiotic resistance. For instance *As* resistance to oxytetracycline, tetracycline and chlorafenicol has been previously reported (Adams et al.

1998, L'Abée-Lund & Sørum 2002, Sørum et al. 2003). Autogenous vaccines against atypical furunculosis are also used in cleaner fish hatcheries as licenced vaccines are not available. Further characterisation of these *vapA* types through partial and / or whole sequencing (e.g. pulsed field electrophoresis; PFGE and next generation sequencing; NGS) can be helpful on identifying differences within the a*As* strains that belong to the same type. This information can then be used to improve/update existing autogenous vaccines.

In addition, interestingly, 47.7% of vaccinated individuals (21 /43 individuals) were positive for the bacterium (a*As*). Given that the majority of the positive individuals (20 / 21) had been vaccinated in the same site (site A), there is a strong suggestion that the vaccination did not appear to prevent infection by a*As* in these fish. Protection may be influenced by the immunisation regime used as well as the isolates included in the vaccine. Both immersion and injection vaccination were being used for ballan wrasse during the time frame of this study but little is known about the efficacy of either administration routes of the vaccine. These findings support the importance for assessing immunocompetence of ballan wrasse and vaccinating the individuals at an appropriate size so that uptake and immune responsiveness to vaccine antigens is optimal. Administration of vaccines at earlier life stages of fish can lead to immunosuppression (Joosten et al. 1995, Covello et al. 2013). The majority of the individuals sampled did not show external/gross signs of disease. However, clinical signs and histopathological changes following infection by the bacterium in ballan wrasse have not yet been described, even though experimental trials have been conducted. For instance, Biering et al. (2016) showed mortalities (75 – 89 % and 51%, respectively) in juvenile ballan wrasse (50 g) infected with a*As* either through intraperitoneal injection or cohabitation. Currently, there are not known reports of disease in farmed Atlantic salmon related with these a*As* strains (type V or VI) and co-infection did not occur during cohabitation with diseased wrasse (Gravningen et al. 1996, Treasurer 2012). Moreover, cultured Atlantic salmon are protected against typical *As* as routine vaccination takes place (Sommerset et al. 2005, Midtlyng 2014).

Bacteria belonging to the *Vibrio* and *Aliivibrio* genus (*V. ichthyoenteri, V. splendidus, V. tasmaniensis, Aliivibrio salmonicida,*) known to be pathogenic to other fish species were recovered from tissue samples of ballan wrasse in this survey in 8 out of 10 sites. *V. splendidus, A. logei, A. wodanis and V. tapetis* have also been isolated from cleaner fish in Norway (Hjeltnes et al. 2018). However, *Vibrios* are universal marine bacteria and three species, *V. splendidus*, *V. ichthyoenteri* and *V. pacinii* may be part of the gut flora of ballan wrasse and goldshinny wrasse (*Ctenolabrus rupestris* L.) (Birkbeck & Treasurer 2014). Thus, isolation of *V. splendidus* and *V. ichthyoenteri* during the survey may have been due to accidental eruption of the gut wall, even though there is not such report. Furthermore, the presence of *Vibrio* species in the organs (liver and kidney) may have occurred at low levels that the immune system could cope with. Nonetheless these bacteria may still pose a threat as opportunistic pathogens for ballan wrasse in commercial production or during stressful events in cage sites. Similarly, ballan wrasse experienced low (10 – 20%) or no mortalities from *V. anguillarum* isolated from ballan wrasse during bath and cohabitation challenge, while i.p. injection of an Atlantic salmon strain was more virulent (50 – 60 %) (Biering et al. 2016). On the other hand lumpsuckers are known to be susceptible to *V. anguillarum*, *V. ordalii* and *V. splendidus* (Bornø & Gulla 2016). Taking the above into consideration, it is not known if ballan wrasse can act as carries of these bacteria and infect lumpfish during cohabitation in sea pens and *vice versa*.

A range of non – pathogenic bacteria known to ballan wrasse were recovered during this study. From those *V. tapetis, T. dicentrarchi and P. indicum/profundum* are worth mentioning. *Vibrio tapetis* is a known pathogen for bivalves, clam species and Atlantic halibut (Reid et al. 2003, Paillard, 2004). Although, juvenile ballan wrasse (approx. 30 g) were not susceptible to these bacteria species during cohabitation challenge and only i.p.

injected shedder fish experienced mortalities (Gulla et al. 2017), it is not known if larvae or younger age juvenile ballan wrasse (<30 g) can be susceptible to the bacteria under rearing conditions. *Tenacibaculum dicentrachi* was isolated from ballan wrasse during this survey and to the best of the author's knowledge this is the first time that *T. dicentrachi* was recovered from ballan wrasse in Scotland. The bacterium belongs to the Family *Flavobacteriaceae* and *Tenacibaculum* spp. are ubiquitous bacteria of the marine environment with a few members of the genus related with fish diseases. For instance, *T. dicentrachi* was first isolated from European sea bass (*Dicentrarchus labrax*) in Spain (Piñeiro-Vidal et al. 2012) and is now a rapidly emerging pathogen of farmed Atlantic salmon in Chile (Avendaño-Herrera et al. 2016). In Norway, isolates of the genus have been recovered from skin ulcers from salmonids and non-salmonid species (Olsen 2017). Understanding the pathogenicity of this bacterium in individual ballan wrasse is important considering that the closely related species of the genus are an emerging bacteria pathogen for salmonids. Cohabitation with diseased salmon can lead to disease transmission between hosts.

Photobacterium indicum/profundum, also reported in this screening, has not previously been associated with fish disease outbreaks but has been isolated from moribund lobster and associated with stress (Basti et al. 2011). A number of isolates (7/151; 6/82 individuals) were recovered from diseased ballan wrasse in this study which might be indicative of a secondary infection after individuals had been infected with a*As*. Recently, *Photobacterium sp*., were recovered from lumpsuckers experiencing mortalities due to *Pseudomonas anguilliseptica* under rearing conditions in Scotland (Treasurer & Birkbeck 2018). Further investigation is needed regarding the pathogenicity and transmission between hosts in order to understand the importance of this bacterium in cleaner fish hatcheries and deployment sites.

Overall, a*As* was the most prevalent bacterial species isolated form ballan wrasse on the farm sites considering the number of individuals sampled in total, followed by *Vibrio* species which were the most geographically prevalent bacteria. Understanding the prevalence of these pathogens is vital for mitigating disease outbreaks by optimising fish husbandry and biosecurity practices. Furthermore, the collection of these bacterial isolates provides useful information for disease management. Also, characterisation of the *aAs vapA* types could provide important information for improving/updating existing autogenous vaccines.

Acknowledgements

The authors would like to thanks Ridgeway Biologicals for samples collection and culture identification and MOWI Scotland and Scottish Sea Farms for their cooperation. This study was funded by the Scottish Aquaculture Innovation Centre (SAIC) under project number SL-2015-01 ‘Sustainable production of wrasse for sea lice control’ (Health WP3).

LITERATURE CITED

Adams CA, Austin B, Meaden PG and McIntosh D (1998) Molecular characterization of plasmid-mediated oxytetracycline resistance in *Aeromonas salmonicida*. Appl Envir Microb 64:4194−4201.

Austin B and Austin DA (2017). Bacterial Fish Pathology. In C. Aeromonadaceae Representative (*Aeromonas salmonicida*), 6th ed, Springer, pp. 216 - 217

Avendaño-Herrera R, Irgang R, Sandoval C, Moreno-Lira P, Houel A, Duchaud E, M Poblete-Morales P, Nicolas P and Ilardi P (2016) Isolation, characterization and virulence potential of *Tenacibaculum dicentrarchi* in salmonid cultures in Chile. Transbound Emer Dis, 63: 121–126.

Basti D, Bouchard D and Lichtenwalner A (2011) Safety of Florfenicol in the Adult Lobster (*Homarus americanus*). J Zoo Wildlife Med 42: 131-133.

Biering E, Krossøy VB, Gulla S and Colquhoun DJ (2016) Challenge models for atypical *Aeromonas salmonicida* and *Vibrio anguillarum* in farmed ballan wrasse (*Labrus bergylta*) and preliminary testing of a trial vaccine against atypical *Aeromonas salmonicida*. J Fish Dis 39:1257–1261.

Birkbeck TH and Treasurer JW (2014) *Vibrio splendidus*, *Vibrio ichthyoenteri* and *Vibrio pacinii* isolated from the digestive tract microflora of larval ballan wrasse, *Labrus bergylta* Ascanius, and goldsinny wrasse, *Ctenolabrus rupestris* (L.). J Fish Dis 37: 69–74.

Brooker AJ, Papadopoulou A, Gutierrez C, Rey S, Davie A and Migaud H (2018) Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Vet Rec 183: 383.

Bornø G and Gulla S (2016) The Health Situation in Norwegian Aquaculture: The Norwegian Veterinary Institute, p. 117.

Daly JG, Kew AK, Moore AR and Olivier G (1996) The cell surface of *Aeromonas almonicida* determines in vitro survival in cultured brook trout (*Salvelinus fontinalis*) peritoneal macrophages. Microb Pathog 21:447– 461.

Gravningen K., Kvenseth PG and Hovlid RO (1996) Virulence of *Vibrio anguillarum* serotypes 01 and 02, *Aeromonas salmonicida subsp. salmonicida* and atypical *Aeromonas salmonicida* to goldsinny wrasse. In: Wrasse Biology and use in Aquaculture (ed. by MDJ Sayer, JW Treasurer and MJ Costello), Blackwell, Oxford, pp. 247–250.

Gulla S, Lund V, Kristoffersen AB, Sørum H and Colquhoun DJ (2016) *vapA* (A-layer) typing differentiates *Aeromonas salmonicida* subspecies and identifies a number of previously undescribed subtypes. J Fish Dis 39:329–42.

Gulla S, Rønneseth A, Sørum H, Vågnes Ø, Balboa S, Romalde JL and Colquhoun DJ (2017) *Vibrio tapetis* from wrasse used for ectoparasite bio-control in salmon farming: hylogenetic analysis and serotyping. Dis Aquat Organ 125:189-197.

Gulla S, Bayliss S, Björnsdóttir B, Dalsgaard I, Haenen O, Jansson E, McCarthy U, Scholz F, Vercauteren M, Verner-Jeffreys D, Welch T, Wiklund T and Colquhoun DJ (2019) Biogeography of the fish pathogen *Aeromonas salmonicida* inferred by *vapA* genotyping. FEMS Microbiol Lett, 366.

Hjeltnes B, Bang-Jensen B, Bornø G, Haukaas A and Walde C S (Ed.) (2018) The Health Situation in Norwegian Aquaculture 2017 Norwegian Veterinary Institute..

Joosten PHM, Aviles-Trigueros M, Sorgeloos P, Rombout JHWM (1995) Oral vaccination of juvenile carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*) with bioencapsulated *Vibrio anguillarum* bacterin. Fish Shellfish Immunol 5:289–299

Kay WW, Buckley JT, Ishiguro EE, Phipps BM, Monette JP and Trust TJ (1981) Purification and disposition of a surface protein associated with virulence of *Aeromonas salmonicida*. J Bacteriol 147:1077–1084.

Kay WW and Trust TJ (1991). Form and functions of the regular surface array (S-layer) of *Aeromonas salmonicida*. Experientia 47:412–414.

Karlsbakk E, Olsen AB, Einen ACB, Mo TA, Fiksdal IU, Aase H, Kalgraff C, Skår SÅ and Hansen H (2013) Amoebic gill disease due to *Paramoeba perurans* in ballan wrasse (*Labrus bergylta*). Aquaculture 412-413: 41 – 44.

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, and Glöckner FO (2012) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41.

L'Abée-Lund TM and Sørum H (2002) A global non-conjugative tet C plasmid, pRAS3, from *Aeromonas salmonicida*. Plasmid 47, 172−181.

Lund V, Espelid S and Mikkelsen H (2003b) Vaccine efficacy in spotted wolffish Anarhichas minor: relationship to molecular variation in A-layer protein of atypical *Aeromonas salmonicida*. Dis Aquatic Organ 56, 31– 42.

Midtlyng PJ (2014) Vaccination against Furunculosis. *In* Fish Vaccination, 1st Edition, Eds. R. Gudding, A Lillehaug, Ø. Evensen, Wiley Blackwell, Ch. 16, pp. 185 – 199.

Munn CB, Ishiguro EE, Kay WW and Trust TJ (1982) Role of surface components in serum resistance of virulent *Aeromonas salmonicida*. *Infection and Immunity* 36:1069–1075.

Olsen AB, Gulla S, Steinum T, Colquhoun DJ, Nilsen H. and Duchaud E (2017) Multilocus sequence analysis reveals extensive genetic variety within *Tenacibaculum spp*. Associated with ulcers in sea-farmed fish in Norway. Vet Microbiol 205: 39–45.

Paillard C (2004) A short review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*. Aquat Living Resour 17:467–475.

Piñeiro-Vidal M, Gijón D, Zarza C and Santos Y (2012) *Tenacibaculum dicentrarchi* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from European sea bass. Int J Syst Evol Micr 62, 425-429.

Reid H, Duncan HI, Laidler A, Hunter D and Birkbeck TH (2003) Isolation of Vibrio tapetis from cultivated Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 221: 65–74

Scholz F, Ruane NM, Morrissey T, Marcos-López M, Mitchell S, O'Connor I, Mirimin L, MacCarthy E and Rodger HD (2018) Piscine myocarditis virus detected in corkwing wrasse (*Symphodus melops*) and ballan wrasse (*Labrus bergylta*). J Fish Dis 41:147 – 152.

Skiftesvik, AB, Bjelland RM, Durif CMF, Johansen IS and Browman HI (2013) Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture 402-403:113–118.

Sommerset I, Krossøy B, Biering E and Frost P (2005) Vaccines for fish in aquaculture. Expert Rev Vaccines 4:89–101.

Sørum H, L'Abée-Lund TM, Solberg A and Wold A (2003) Integron-containing IncU R plasmids pRAS1 and pAr-32 from the fish pathogen *Aeromonas salmonicida.* Antimicrob Agents Ch 47:1285−1290.

Treasurer JW (2012) Diseases of north European wrasse (*Labridae*) and possible interactions with cohabited farmed salmon, *Salmo salar* L. J Fish Dis 3:555–562.

Treasurer JW and Birkbeck H (2018) *Pseudomonas anguilliseptica* associated with mortalities in lumpfish reared in Scotland. B Eur Assoc Fish Pat 38:2018.

Udey JL and Fryer LR (1978) Immunization of fish with bacterins of *Aeromonas salmonicida*. Mar Fish Rev 40:12–17.

Yamamoto S, Kasai H, Arnold DL, Jackson RW, Vivian A and Harayama S (2000) Phylogeny of the genus *Pseudomonas*: intrageneric structure reconstructed from the nucleotide sequences of *gyrB* and *rpoD* genes. Microbiology 146: 2385 – 2394.

Table 1 List of primers used for bacteria species identification.

Primer	Primer name	Target gene	Annealing (°C)	Application	Reference
CAGGAAACAGCTATGACCAYGSNGGNGGNAARTTYRA	UP -1E	*gyrB*	60	PCR	Yamamoto et al., 2000
TGTAAAACGACGGCCAGTGCNGGRTCYTTYTCYTGRCA	APrU				
AGAGTTTGATCMTGGC	Bact-0008	16S	44	PCR	Klintworth et al., 2013
CCGTCAATTCMTTTGAGTTT	Bact-0907				
CTGGACTTCTCCACTGCTCA	F2	*vapA*	53	PCR and sequencing	Lund et al., 2003b
ACGTTGGTAATCGCGAAATC	R3				Gulla et al., 2016

Table 2. Standard bacteriology tests (Gram stain, shape, catalase, oxidase) on pathogenic bacteria isolated from skin lesions, gills, liver and kidney swabs of moribund or recovered ballan wrasse (*Labrus bergylta;* >0.5 to 900 g) during disease outbreaks in hatcheries and salmon sea cage sites in Scotland between February 2016 and October 2018. Sequencing similarity represents blasts results from *16S* and *gyrB* sequencing.

Bacteria species	**Shape**	**Catalase (-/+)**	**Oxidase (-/+)**	**Sequencing similarity (%)**
atypical *Aeromonas salmonicida*	Bipolar rods	-	+	99-100
Vibrio spp.	Curved rods	+	+	99-100
Vibrio (Allivibrio) salmonicida	Curved rods	+	+	99-100
Vibrio tasmaniensis	Rods	+	+	99
Vibrio splendidus	Short rods	+	+	99-100
Vibrio logei	Cigar like rods	+	-	99
Vibrio splendidus	Rods	+	+	99-100
Vibrio ichthyeoenteri	Thin rods	+	+	96-100
Vibrio sp	Rods	+	+	100
Vibrio anguillarum	Bipolar rods	-	+	99
Tenacibaculum dicentrachi	Curved rods	+	+	99
Tenacibaculum solea	Slender rods	-	+	100
Tenacibaculum ovoliticum	Filamentous rods	+	-	100
Pseudomonas spp.	Short bipolar rods	+	+	99-100
Pseudoalteromonas spp.	Bipolar rods	-	+	100
Moritella viscosa	Slightly curved rods	+	+	99
Flavobacterium frigidarium	Chaining cocci-bacillus	+	-	97-100

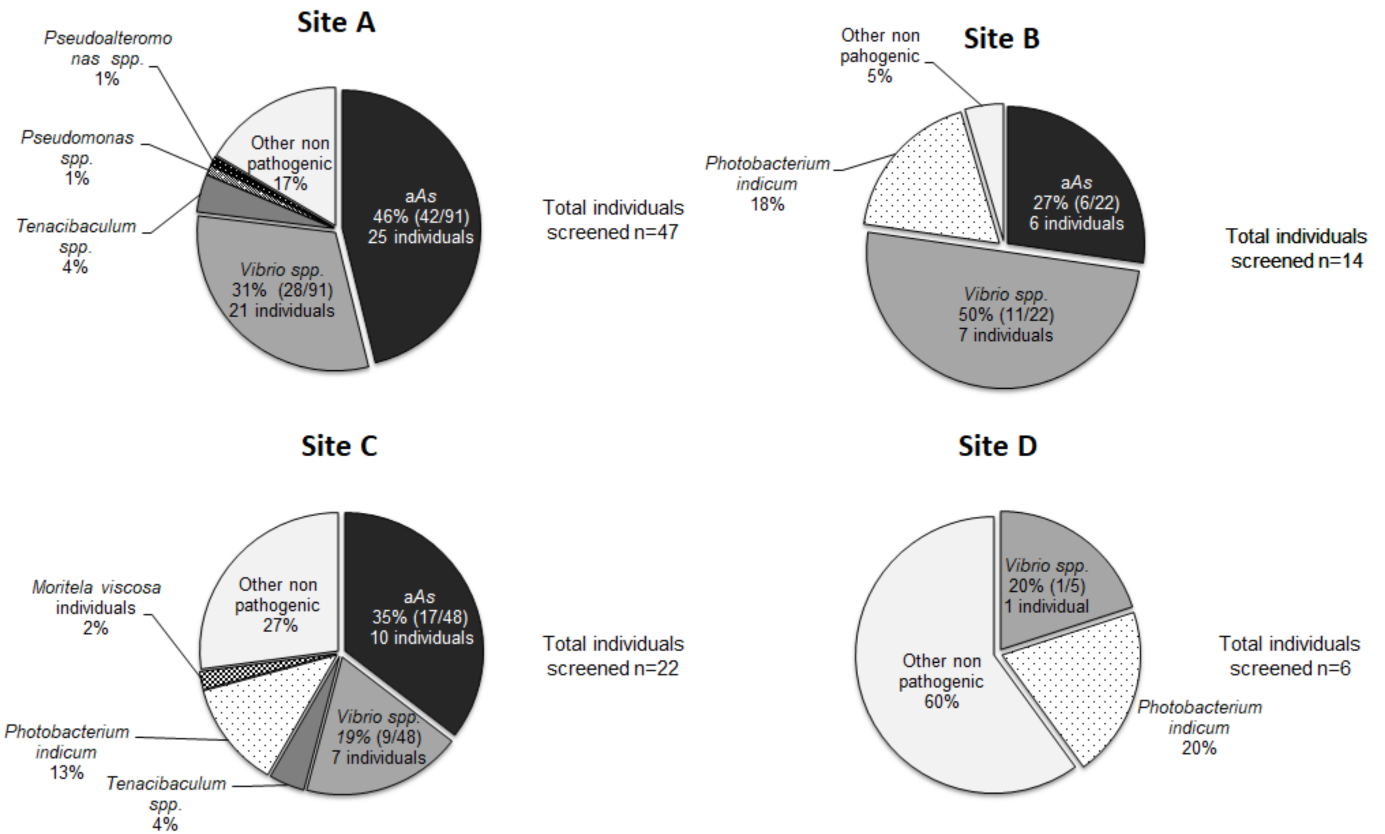

Figure 1. Bacteria percentage recovery from 4 out of 10 sites during a health screening survey for ballan wrasse in Scotland between 2016 and 2018 (Part 1). Swabs from skin lesions, gills, liver and kidney plated on variety of agar plates (Marine Agar, Tryptone Soya Agar (TSA), and TSA + 5%

Defibrinated Horse Blood + 1.5% NaCl) for phenotypic and sequencings identification. Pie chart for Site E is not shown as only atypical *Aeromonas salmonicida* was isolated in a single sampling (2/2). Site F, single sampling point with no bacteria recovery from individuals sampled.

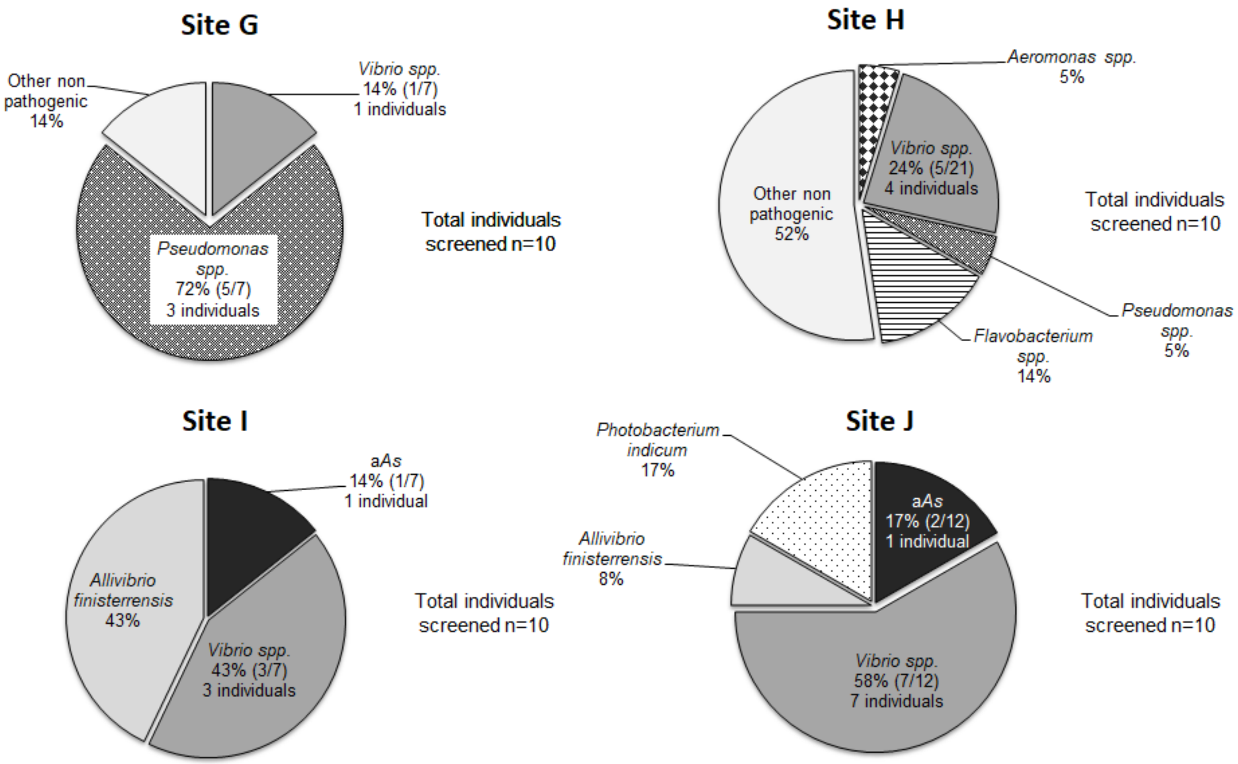

Figure 2. Bacteria percentage recovery from 4 out of 10 sites during a health screening survey for ballan wrasse in Scotland between 2016 and 2018 (Part 2). Swabs from skin lesions, gills, liver and kidney plated on variety of agar plates (Marine Agar, Tryptone Soya Agar (TSA), and TSA + 5% Defibrinated

Horse Blood + 1.5% NaCl) for phenotypic and sequencings identification. Pie chart for Site E is not shown as only atypical *Aeromonas salmonicida* was isolated in a single sampling (2/2). Site F, single sampling point with no bacteria recovery from individuals sampled.